Assessment of Aging of the Human Skin by In Vivo Ultrasonic Imaging

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The ultrasonic imaging technique that we have developed provides cross-sectional images of human skin in vivo with a resolution of about 80 μm axially (i.e., deep into the skin) and 250 μm lateral (parallel to the surface). In order to study aging skin, we obtained ultrasonic images from the mid-forearm (volar and dorsal sides) of 142 women. Ultrasonically, on the images, the dermis appears composed of two bands: a dark superficial one where the ultrasonic waves are propagated in a relatively homogeneous or non-echogenic medium, and a deeper one, which is lighter in color, suggesting a heterogeneous medium. Our results show that skin is thicker on the dorsal than on the volar forearm. In contrast to previously published results, skin thickness remains constant until the seventh decade of life, diminishing thereafter. The relative thicknesses of the two bands show marked variations with age: a progressive thickening of the dark band, from zero in infants to approximately 75% of total skin thickness in aged subjects, while the light band shows the inverse trend. Comparing the amplitude of the bands on the volar and dorsal forearm, the relative thickness of the dark band is larger on the dorsal (exposed) side and increases with age. These findings and the analysis of variously stained biopsies taken in some of our patients lead us to assign this dark band to a zone in the upper dermis where the collagen network is delicate, dense, and well organized. This is supported by some data in the literature. The thickness of this subepidermal non-echogenic band appears to be a far more sensitive marker of skin aging at the dermal level than is the measurement of skin thickness. *J Invest Dermatol 93:621 – 625, 1989*

Human skin aging is accompanied by many clinical signs, the most evident of which are dryness, color changes (yellowing, uneven pigmentation), wrinkles, and a loss of firmness. These modifications play an important role in our perception and estimation of age. Signs of skin aging appear first in the exposed areas (face, hands, chest) where certain clinical anomalies such as actinic keratosis or solar elastosis, directly due to exposure to UV radiation, can also appear. These clinical manifestations parallel morphologic and structural changes in the skin and are likely related to functional declines. The quantitative description of the biochemical modifications in the main constituents of the skin, together with the disturbances that occur in their organization, have been the purpose of numerous studies. Similarly, the functional alterations in the skin with age have been extensively studied, often by means of non-invasive biophysical techniques. Without going into the details of these numerous publications, we would like to underline some general features that explain why, despite this abundant literature, we still remain unable to clearly explain the various phenomena occurring during the skin aging process. First, the studies undertaken have too often been dealing with the effects of environmental factors rather than the physiologic process of aging. Second, the extreme diversity in the techniques used has frequently led to contradictory results [1].

With regard to studies dealing with the skin structure, too little attention has been paid to the gross changes in the skin structures, which, we believe, is essential for understanding the properties of the skin, while there has been much work on the microscopic organization of the skin or on the ultrastructure of the collagenous component. Finally, it is well known that individual variability increases with age; there is therefore a statistical aspect involved which, no doubt, has contributed to the discrepancies observed in some results. The present study describes a new investigation of the skin as a function of age and attempts to avoid some of the pitfalls listed above. It uses a non-invasive biophysical method, B-scan ultrasonic echography, which is a simple and relatively recent technique [2] for producing cross-sectional images of the human skin. Our imaging prototype produces images representing a cross-section of the skin: 2 cm long over 4 mm deep into the skin with a resolution of approximately 80 μm in deepness. A number of structural elements can be identified and studied on the images [3]. The results are extremely consistent from one observer to another. In order to evaluate the possible influence of the environment on the natural process of skin aging, we compared the images of the volar (protected) and dorsal (exposed) sides of the forearm of 142 women.

MATERIALS AND METHODS

Equipment The use of pulsed ultrasound in dermatology is now well known and has been extensively used since 1982 [4]. The incident ultrasonic energy, partially transmitted and partially reflected at boundary between adjacent structures, generates echos, the amplitude of which is characteristic of the nature of the two

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Abbreviations:
DEB: dermal echogenic band
MHz: megahertz
SC: stratum corneum
SENEB: sub-epidermal non-echogenic band

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media. The A-scan signal, obtained at one point, is made of a series of echoes of various amplitude, characteristic of the interfaces encountered.

The acquisition of successive A-scan lines and the conversion of the amplitudes of the rectified signal into grey levels, allows us to form a cross-sectional image of the skin. A heterogeneous medium generates numerous echoes and appears light on the picture. On the contrary, a homogeneous medium does not generate echoes and appears dark. The main feature of our prototype ultrasonic imaging apparatus [5] lies in the large improvement in axial (i.e., deep into the skin) and lateral (i.e., parallel to the surface of the skin) resolution over the commercially available ultrasound echographs used, up to now, in medical research. Our prototype digitizes 100 radiofrequency signal lines produced by a transducer with a central frequency of 25 MHz, focused at 25.4 millimeters, giving an axial resolution of approximately 80 μm and a lateral resolution on the order of 250 μm. Taking into account this latter parameter, the probe is set to move by 0.2 mm steps, giving an effective range of 20 mm. The transducer is housed in a perspex tip filled with an aqueous coupling gel.

All the acquisitions are carried out in a standardized way (same gain and image processing), allowing subject to subject comparison.

Measurement Protocol Each image obtained on the video monitor is photographed in black and white using a standardized procedure. The thickness of the various visible "bands" is measured directly in millimeters on the photograph, taking into account the velocity of ultrasonic waves through the skin (1605 m/s) [6]. Each value represents the mean of six determinations regularly spaced.

Subjects The study concerned the volar and dorsal sides of the mid-region of the forearm of 142 women, with 10 to 20 (mean 15) subjects in each decade of life (0–10 years up to 80–90 years).

Biopsies Punch biopsies (3 mm) were taken on the mid-dorsal forearm of three subjects after informed consent. The histologic sections were stained using Eosin/Hematoxylin and Orcein and Luna technique for Elastin.

Statistics All the results are expressed as mean ± standard error of the mean for each decade and each side studied. Linear regression analysis was performed using the least-square method on the overall experimental data. Analysis of variance was carried out on the experimental data taking into account the age distribution, in decades.

RESULTS

Figure 1*a–d* shows typical ultrasonic skin images obtained on the volar forearm of persons aged 6 years *(a)*, 31 years *(b)*, 49 years *(c)*, and 85 years *(d)*. The skin clearly appears located between the white line produced by the interface between the coupling gel and the stratum corneum (SC) and the more-or-less uniform black area representing the subcutaneous tissue. The junction between the dermis and the underlying fatty tissue is less regular and well-defined than the gel-SC interface. These images show the general trend in skin thickness with age: relatively thin in both children and aged subjects and somewhat thicker in adults. In addition, one can immediately observe below the white line, corresponding to the entry echo, a black zone, previously described [3], corresponding in these conditions to a relatively homogeneous, non-echogenic structure that we refer to as the Sub-Epidermal Non-Echogenic Band (SENEB). It has

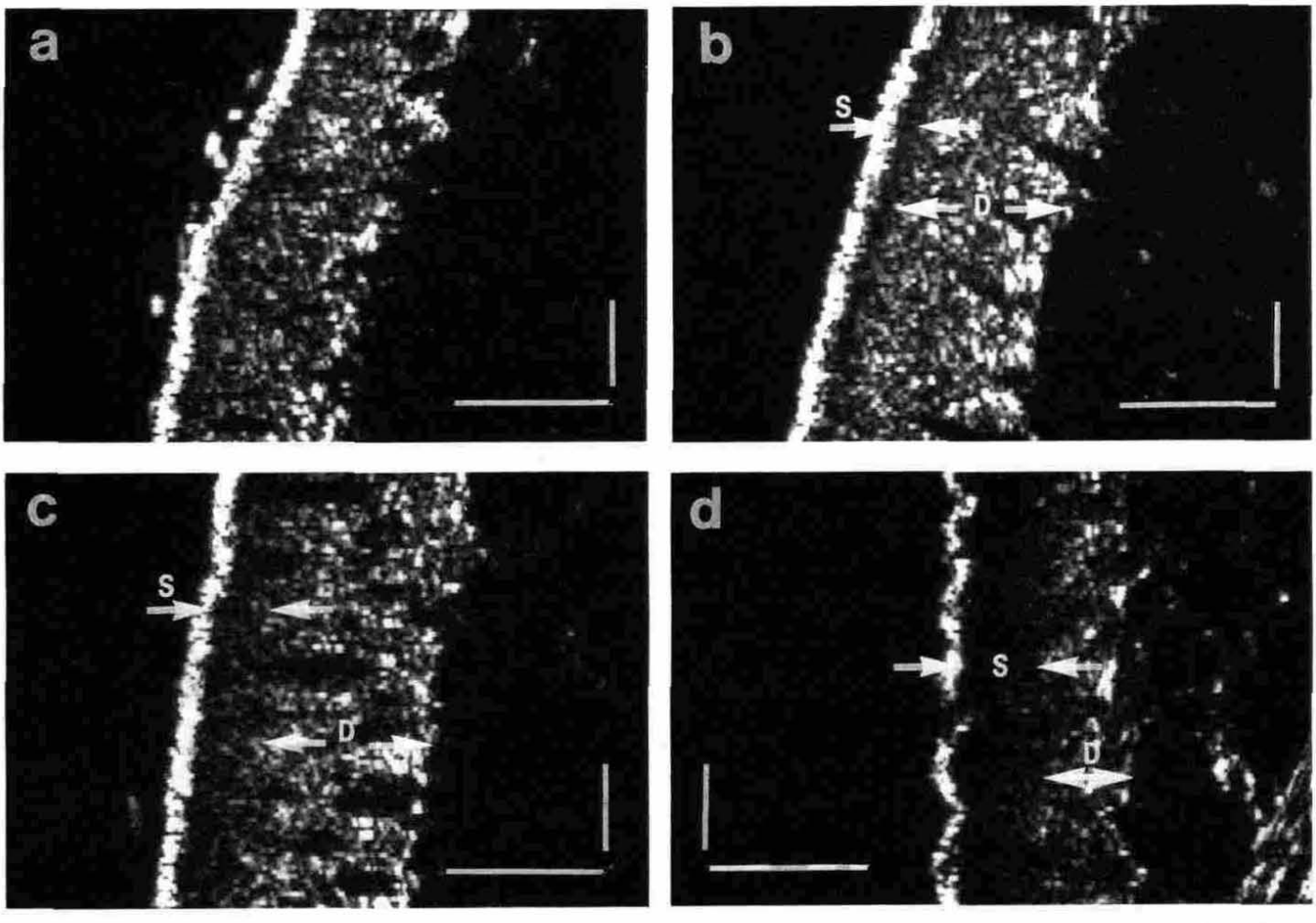

Figure 1. High resolution ultrasonic images of the human skin from *(a)* a child (6 years), *(b)* a young adult (31 years), *(c)* an older adult (49 years), and *(d)* an aged woman (78 years). *Vertical bar:* 5 mm; *Horizontal bar:* 1 mm; *S*: SENEB; *D*: DEB.

been previously checked that the thickness of this band is not influenced by the amplitude of the first major echo, generated by the interface between the gel and the Stratum Corneum.

This band, which is not observed in the young, increases with age and represents in the aged almost the whole thickness of the skin (Fig 1*d*). In contrast, the echogenic band, characteristic of the reticular dermis [3], the Dermal Echogenic Band (DEB), diminishes with age.

Skin Thickness The changes in skin thickness as a function of age in the volar and dorsal forearm are shown in Fig 2. There is a highly significant ($p < 0.001$) difference between these sides. The dorsal skin is approximately 17% thicker than the ventral one; however, both are not different beyond 70 years of age.

On the volar forearm, skin thickness does not vary significantly between the first and seventh decade of life ($p < 0.001$), but atrophy appears ($p < 0.05$) after the eighth decade. Trend of dorsal skin thickness with age is somewhat different. A phase of maturation is observed up to 15 years of age ($p < 0.05$), and atrophy begins after the seventh decade ($p < 0.05$). Analysis of variance detects an age-side interaction ($F = 3.07$, $p < 0.003$) confirming the different behaviors of the two sides (volar and dorsal forearm) with age.

Thicknesses of the Different Bands The age relationship of the thickness of the skin, the SENEB, and the DEB versus age are shown in Fig 3*a* (volar forearm) and 3*b* (dorsal forearm).

On the volar forearm, the variation of the thickness of the SENEB follows a linear equation ($Ep = 0.03 + 0.0048 \times Age$) ($R = 0.844$, $p < 0.0001$). The same holds true for the DEB ($R = -0.454$, $p < 0.001$). With regard to the dorsal forearm, here again, the SENEB is better correlated with age ($Ep = 0.03 + 0.0063 \times Age$) ($R = 0.851$, $p < 0.0001$) than DEB ($R = -0.653$, $p < 0.001$).

Figure 4 shows that, as a whole, the relative thickness of the SENEB is higher in the dorsal than in the volar forearm ($F = 23.3$, $p < 0.001$). However, the class-by-class differences are only significant in the last 3 decades ($p < 0.05$). Moreover, relative thickness of the SENEB increases more rapidly in dorsal than in volar forearm ($F = 4.82$, $p < 0.0001$). In addition, standard error in the last 2 decades are obviously larger than in the preceding ones.

DISCUSSION

Though our results confirm the difference in skin thickness between the dorsal and the volar forearm, they differ from those previously published by Tan et al [7] and Escoffier et al [8] concerning skin thickness as a function of age. These authors both carried out A-scan ultrasound measurement on the forearm. Our results show a skin thickness 15% greater on average than that found in these two studies. By obtaining images of the skin, it can be seen that the skin is not limited by two well defined lines. Although the gel-stratum corneum interface is well characterized, the limit of the dermis is difficult to delineate. In the echographic A-scan technique, it is therefore difficult to determine which echo exactly corresponds to the dermis-hypodermis interface. In the B-scan technique, this interface is more precisely determined directly on the picture where the doubt on one line is removed by the preceding or the following one. In addition, skin thickness determination by A-scan technique is based on the selection of the last major echoe

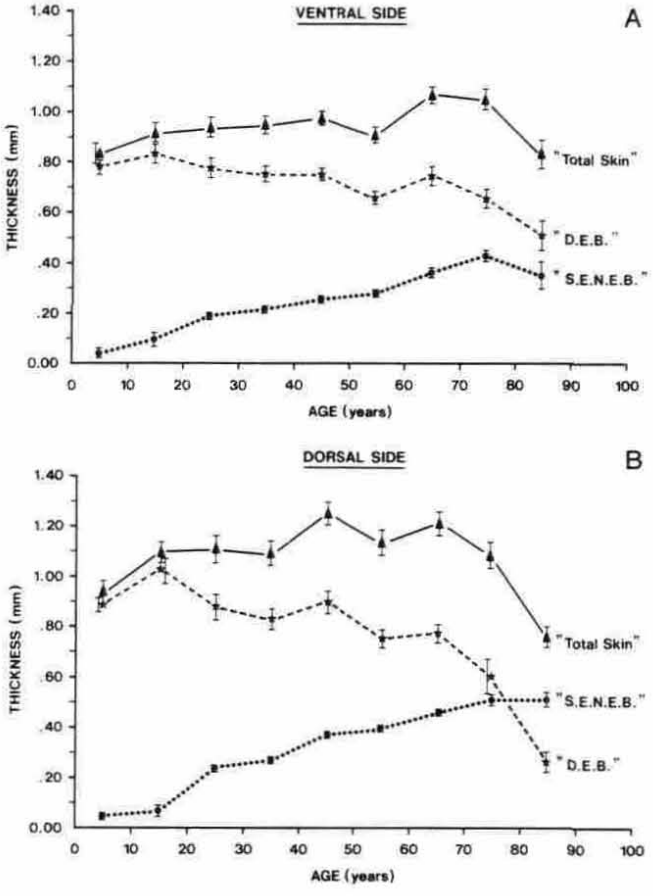

Figure 3. Variation with age of the thicknesses of SENEB, DEB, and total skin. *a*: Ventral side; *b*: dorsal side.

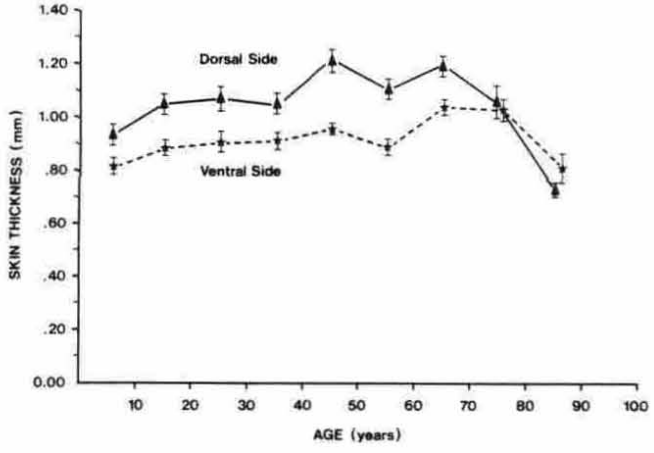

Figure 2. Variation of skin thickness (forearm) with age. Dorsal side: *solid line;* ventral side: *dashed line.*

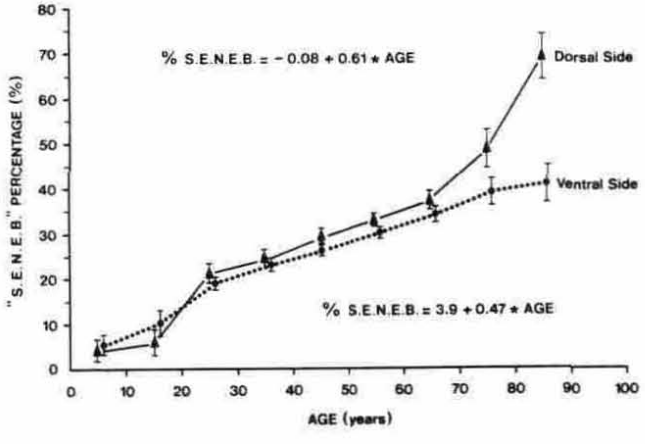

Figure 4. Variation with age in the relative contribution (%) of the SENEB to the total skin thickness. Ventral side: *dashed line;* dorsal side: *solid line.*

induced by the dermis-hypodermis interface. In B-scan, the interface is delineated by the last of all the echoes, related to the skin structure. Differences in the population cannot explain these discrepancies because the results obtained by these two authors are indeed superimposable, although the populations studied were different.

The problem concerning the progressive reduction in skin thickness with age has been discussed elsewhere [8,9]. Is this reduction continuous after 30 years of age [7], or is skin thickness relatively constant until approximately 65 years, becoming thinner thereafter [8]? A comparative study of these results favors the second hypothesis [9].

The most striking result of the present study is the marked variation with age of the two structural elements that this new technology is able to distinguish. As can be seen in Figs 3*a,b*, these two structural elements inversely change with age. The SENEB, hardly visible in the child, represents nearly 75% of total skin thickness in the aged subject, whereas the thickness of the DEB decreases almost continuously. The interindividual variations of the percentage of the SENEB in the skin are larger in the old groups than in the young groups, a frequent phenomenon observed in studies on skin aging. These large and continuous age-related changes raise the question of the actual anatom identities of these two bands.

In the ultrasonic technique, the signal received by the detector and converted into grey levels corresponds to echoes produced by partial reflection of ultrasonic waves at the interfaces of media with different acoustic properties. A black zone therefore corresponds to a homogeneous or near-homogeneous medium composed of structural elements whose spacial periodicity is greatly inferior to the wavelength used (60 μm under our experimental conditions). A white or grey zone corresponds to medium composed of more heterogeneous structures forming a multitude of interfaces at distances superior or of the same order as the wavelength used. In a previous work, Querleux et al [3], studying an adult population interpreted the SENEB as image of the adventitial dermis. The nature of this upper or papillary dermis, a well-vascularized area composed of thin collagen bundles, corresponds to the ultrasonic criteria of a homogeneous and therefore non-echogenic medium. In contrast, the reticular dermis, composed of large collagen bundles, would correspond to the DEB. It is logical to interpret the ultrasonic images that reveal gross structural elements in terms of the density and organization of the collagen [11], the principal component of the dermis (approximately 60% of the dry weight). Recent work has shown that the size distribution of the collagen bundles varies with their location within the dermis [11,12]. In the upper dermis, they appear thin, forming a "feltwork" composed of thin bundles closely interwoven, while the reticular dermis, composed of larger wavy bundles, loosely interwoven, contains numerous voids filled by hydrated proteoglycans or glycosaminoglycans.

This can be observed in Fig 5, which shows a cross-section of the skin of a 37-year-old subject: the histometric differences in the bundles clearly appear according to their location within the dermis [superficial (Fig 5*a*), central, or deep (Fig 5*b*)]. The progressive increase in the amplitude of the SENEB with age could therefore reflect a relative increase in thin collagen bundles with regard to larger ones. This is confirmed by optical microscopic examination of histologic cross-sections in agreement with the findings of Lovell [12]. Other authors, including Lavker [11], reported that in the child the papillary dermis was hardly distinguishable from the reticular dermis. This finding fits with the absence of SENEB in the skin images of children. However, a more precise interpretation of this SENEB needs deeper structural investigations.

In Fig 4, it can be clearly seen that the SENEB thickness is related to light exposure. It has been recently shown that solar damaged skin displays a characteristic low dermal echo amplitude, as observed by A-scan ultrasound echography [13]. This would corre-

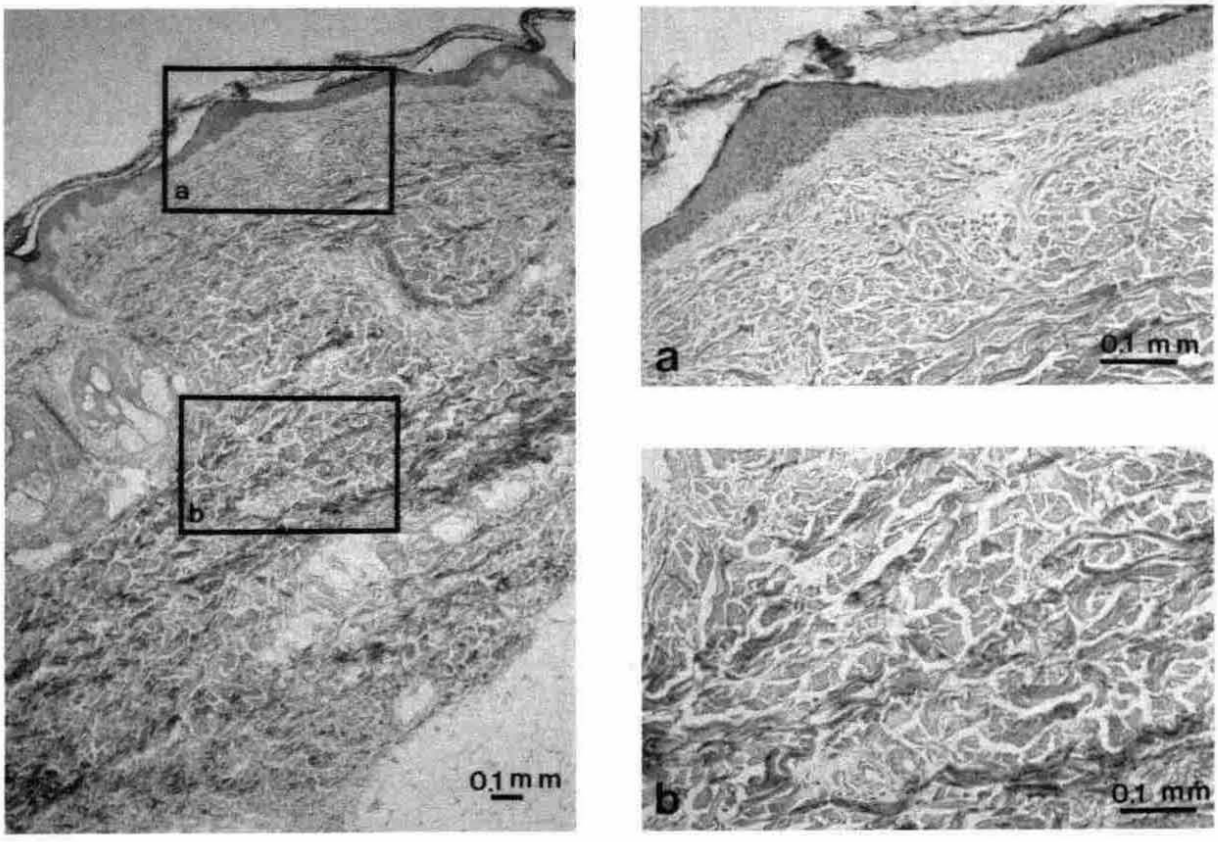

Figure 5. Histology of the skin forearm of a 37-year-old woman (Hematoxylin/Eosin staining). *a*: Epidermis and upper part of the dermis; *b*: lower part of the dermis.

spond to the presence of the SENEB in the B-scan technique. To check this hypothesis, histologic examinations were carried out on three subjects having a significative SENEB (30% to 70% of the total skin thickness). Both volar and dorsal forearm show that elastotic material was only detectable in the form of thin fractionated fibers. We therefore consider that elastotic material is not the unique cause of the reduction in the echo amplitude leading to the SENEB. This can also be due to collagen bundles too thin and too dense to be detected by the ultrasonic waves with a resolution of approximately 80 μm.

The skin has often been described by various authors as a highly resistant organ whose fundamental properties remain intact throughout most of the life span. This view is supported by results such as those concerning skin thickness and extensibility [8]. However, recent measurements of mechanical properties as a function of age have shown a relatively rapid and progressive decline in the ability of the skin to compensate the deformation [8,14]. The present study also highlights important changes in the structure of the skin which in turn might also explain the results obtained concerning its elasticity. Thus, in terms of elasticity and ultrasonic imaging, the skin appears to be an organ whose structure, composition, and some functions begin to change immediately following maturity. This is in agreement with the opinion that some specialists have repeatedly stated [15]. Clearly, the relative thickness of the SENEB appears to be a more sensitive parameter for skin aging than the total skin thickness.

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